

Appl. No. 10/538,840  
Amendment dated: September 9, 2009  
Reply to OA of: June 9, 2009

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims:**

**Claims 1-26 (Canceled)**

27. (Previously Presented) An isolated polynucleotide which encodes for a protein with trans-sialidase activity, wherein said polynucleotide can be isolated from *Trypanosoma congolense* and which comprises one of the nucleic acid sequences selected from the group consisting of SEQ ID NO: 1 and 3; the polynucleotides complementary to the same; or nucleotide sequences differing from said polynucleotides by degeneration of the genetic code.

28. (Previously Presented) The isolated polynucleotide of claim 27, which encodes for a protein with trans-sialidase activity and catalyzes the transfer of sialic acid from a donor onto an acceptor molecule.

29. (Previously Presented) An isolated oligonucleotide, which hybridizes with a polynucleotide of claim 27 or 28 under stringent conditions comprising washing at 20-25°C for 5-10 minutes with 2xSSC buffer containing 0.1 % SDS and a subsequent washing with a buffer of 0.1 x SSC buffer with 0.1 % SDS, at a temperature of 64°C.

30. (Previously Presented) An isolated polynucleotide, which hybridizes with an oligonucleotide of claim 29 under stringent conditions, comprising washing at 20-25°C for 5-10 minutes with 2xSSC buffer containing 0.1 % SDS and a subsequent washing with a buffer of 0.1 x SSC buffer with 0.1 % SDS, at a temperature of 64°C, and encodes for a gene product of microorganisms of the *Trypanosoma* genus.

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31. (Currently Amended) An isolated polypeptide, which is encoded by the isolated polynucleotide of claim 27 or 28, wherein the isolated polypeptide is encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 or 3.

32. (Previously Presented) An isolated trans-sialidase obtainable from *Trypanosoma congolense*, characterized by one of the following amino acid part sequences:

TDTVKYSTDGGRTWKREVIIPNGR (pos. 1 to 25 of SEQ ID NO: 2) or

FRIPSLVEIDGVLIATFDTRYLRASDSSLI (pos. 1 to 30 of SEQ ID NO: 4).

33. (Previously Presented) The isolated trans-sialidase of claim 32, wherein the isolated trans-sialidase consists of the amino acids of SEQ ID NO: 2 and is characterized by at least one of the following characteristics:

- i) Temperature optimum 30-40°C
- ii) pH optimum pH 6.5-8.5
- iii) Isoelectric point pH 4-5
- iv) Molecular weight, native 400-600 kDa
- v) Molecular weight in the reducing SDS page 90 kDa .

34. (Previously Presented) The isolated trans-sialidase of claim 32, wherein the isolated trans-sialidase consists of the amino acids of SEQ ID NO: 4 and is characterized by at least one of the following characteristics:

- i) Temperature optimum 30-40°C
- ii) pH optimum pH 6.5-8.5
- iii) Isoelectric point pH 5-6
- iv) Molecular weight, native 120-180 kDa
- v) Molecular weight in the reducing SDS page 90 kDa .

35. (Previously Presented) The isolated polynucleotide of claim 27, isolated from the *Trypanosoma congolense* organism.

36. (Canceled)

37. (Canceled)

38. (Previously Presented) An isolated nucleotide sequence, encoding a trans-sialidase of claim 32.

39. (Previously Presented) An expression cassette, comprising, operatively linked to with at least one regulative nucleic acid sequence, a nucleic acid sequence of claim 38.

40. (Previously Presented) A recombinant vector, comprising at least one expression cassette of claim 39.

41. (Previously Presented) Prokaryotic or eukaryotic host, transformed with at least one vector of claim 40.

42. (Currently Amended) A method for the enzymatic sialization of an acceptor molecule, characterized in that the acceptor molecule is incubated with a donor containing sialic acid residues in the presence of an enzyme of claim [[31]] 32, and the sialylated acceptor is isolated.

43. (Previously Presented) The method of claim 42, characterized by at least one more of the following properties:

- a) the donor is selected from the group consisting of sialic acids bonded to oligosaccharides, polysaccharides, polysialic acids, glycoproteins and glycolipids.
- b) the acceptor is selected from the group consisting of polymers containing  $\beta$ -

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galactose, such as  $\beta$ -galactooligosaccharides, lactitol, lactobionic acid, methyl- $\beta$ -lactoside, acetyllactosamines, galactopyranosides, trans-galactooligosaccharides, polygalactose and other glycoconjugates with terminally bonded  $\beta$ (1-3) or  $\beta$ (1-4) galactose or galactose.

**44. (Canceled)**

45. (Previously Presented) A method for the isolation of an enzyme with trans-sialidase activity as defined in claim 32, comprising:

*cultivating Trypanosoma congolense* in a medium so that said *Trypanosoma congolense* expresses the trans-sialidase,

obtaining a culture supernatant containing said trans-sialidase, and

isolating the trans-sialidase from the culture supernatant with exchange chromatography by applying a salt gradient.

46. (Previously Presented) The method of claim 45, additionally comprising isoelectric focussing, gel filtration, affinity chromatography and/or protein precipitation.

**47. (Canceled)**

48. (Previously Presented) A foodstuff or food additive comprising the isolate of claim 32.